

SEQUENCE MATCHING, SIMPLE SEARCHING

PGA Course in Bioinformatics
Tools for Comparative Analysis

July 15, 2002

Outline

☛ Sequence alignment algorithms

- Rigorous Optimality: Needleman-Wunsch and Smith-Waterman
- Rapid, heuristic algorithms
 - BLAST
 - FASTA
 - and their relatives

☛ Databases and Search Tools

MAJOR SITES WE WILL USE

☞ <http://www.ncbi.nlm.nih.gov/>

☞ <http://workbench.sdsc.edu>

Needleman Wunsch Algorithm

- ☞ Global alignment:: every residue of the two sequences has to participate
- ☞ Guaranteed to calculate an Optimal similarity score
- ☞ Begin at the beginning of each sequence and go to the end.
- ☞ Cannot detect domains

Smith-Waterman Algorithm

- Optimal Local Alignment
- Guaranteed to find all significant matches to a given query
- Takes the query sequence versus every sequence in the database
- Can be used with arbitrary scoring systems
- **COMPUTATIONALLY EXPENSIVE!!!**

Scoring Matrices

- Relatively simple for DNA-gap penalties or mismatches-can be made to look at Pu/Py
- Protein matches look also at similarity (leu/ileu)

Protein Scoring Matrices

- ☛ Chemical similarity: 210 pairs of aa
- ☛ Nearness in Genetic Code
- ☛ Chemical similarity, e.g.,
hydrophobicity
- ☛ Observed Substitution Schemes

Observed AA Substitution Matrices

- ☛ PAM
- ☛ BLOSUM

PAM: Point Accepted Mutation

- ☛ DAYHOFF et al.
- ☛ Observed residue replacement in related proteins
- ☛ GLOBAL alignment, closely related
- ☛ A model of molecular evolution
- 1 PAM = average change in 1% of all amino acid possibilities (1% divergence)
- ☛ Other PAM matrices extrapolated from PAM1.

PAM continued

- ☛ TIME is NOT correlated with PAM
- ☛ Number of the matrix refers to evolutionary distance

Means different families of proteins evolve at different rates

PAM250

Table 2: The PAM250 matrix – an example of a matrix derived from observed substitutions

	A	B	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Z
A	2	9	-1	0	0	-4	1	-1	-1	-2	-1	0	1	8	-2	1	1	8	-4	-3	0	
B	0	2	-6	2	3	-5	9	1	-2	1	-2	2	-1	1	-1	8	0	-3	-5	-1	2	
C	-2	-4	12	-5	-3	-4	-3	-2	-2	-5	-6	-5	-4	-3	-3	0	-2	-8	-6	-5	0	
D	0	3	-5	6	3	-4	1	1	-2	6	-4	-2	2	-1	2	-1	0	9	-2	-7	-6	3
E	8	3	-5	3	6	-5	9	1	-2	0	-3	-2	1	-1	2	-1	0	9	-2	-7	-6	3
F	-1	0	-2	1	0	-3	2	-1	-3	-2	-4	-3	0	-1	-1	-2	1	8	-6	-7	-5	-1
G	-1	1	-2	1	1	-2	-2	4	-2	0	-2	-2	3	0	2	-1	-1	-2	-3	0	2	
H	-1	1	-2	1	1	-2	-2	4	-2	0	-2	-2	3	0	2	-1	-1	-2	-3	0	2	
I	-1	-2	-3	-2	-3	1	-1	-2	3	-2	3	-2	-2	-2	-2	-3	0	6	-5	-1	-2	
K	-1	1	-2	0	0	-2	-2	0	-2	5	-3	8	1	-1	1	0	0	-2	-3	-4	0	
L	-2	-3	-4	-3	-2	-4	-2	2	-3	4	4	-3	-3	-3	-3	-3	-2	3	-2	-1	-2	
M	-1	-1	-3	-2	3	-2	2	8	4	-3	-3	-1	8	-2	-2	2	-4	-2	-2	-2	-2	
N	0	3	-4	2	1	-4	2	-2	1	-3	-2	2	-1	1	0	1	9	-2	-4	-2	1	
P	1	-1	-2	-1	-2	-3	-1	8	-3	-1	-2	-2	1	8	0	0	1	9	-1	-6	-5	8
Q	8	1	-5	2	2	-5	-1	3	-2	1	-2	-1	1	0	4	1	-1	-2	-5	-4	3	
R	-2	-1	-3	-1	-1	-4	-2	2	-2	3	-3	8	0	0	1	4	9	-1	-2	2	-4	6
S	1	0	8	0	8	-3	1	-1	0	-2	-2	1	-1	0	2	1	-1	-2	-3	0	1	
T	1	8	-2	0	8	-3	0	1	0	-2	-1	0	9	-1	-1	1	3	8	-3	-1	-1	
V	8	-3	-2	-2	-2	-1	-2	6	-2	2	1	-2	-1	-2	-2	-1	0	4	-6	-3	-2	
W	-4	-5	-6	-7	-7	0	-7	-5	-3	-2	-6	-4	-3	-3	-2	-5	-4	17	6	-6	-6	
Y	-3	-1	0	-4	-6	7	-5	0	-1	-4	-1	-2	-1	-4	-6	-3	-3	-2	0	20	-4	
Z	0	2	-5	3	3	-6	-1	2	-2	6	-3	-2	1	6	3	0	-1	-2	-6	-4	3	

The row and column letters and the numbers refer to the 20 amino acids: A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y, Z.

Blosum62

	A	C	D	E	F	G	H
A	4	0	-2	-1	-2	0	-2
C	0	9	-3	-4	-2	-3	-3
D	-2	-3	6	2	-3	-1	-1
E	-1	-4	2	5	-3	-2	0
F	-2	-2	-3	-3	6	-3	-1
G	0	-3	-1	-2	-3		
H	-2	-3	-1				

BLOSUM 62

BLOSUM

- ☛ Block Substitution Matrix
- ☛ Henikoff and Henikoff, PNAS, 1992
- ☛ Number following indicates per cent identity within set, BLOSUM62=62% id
- ☛ Finds short, highly similar sequences (no gaps)

BLOSUM

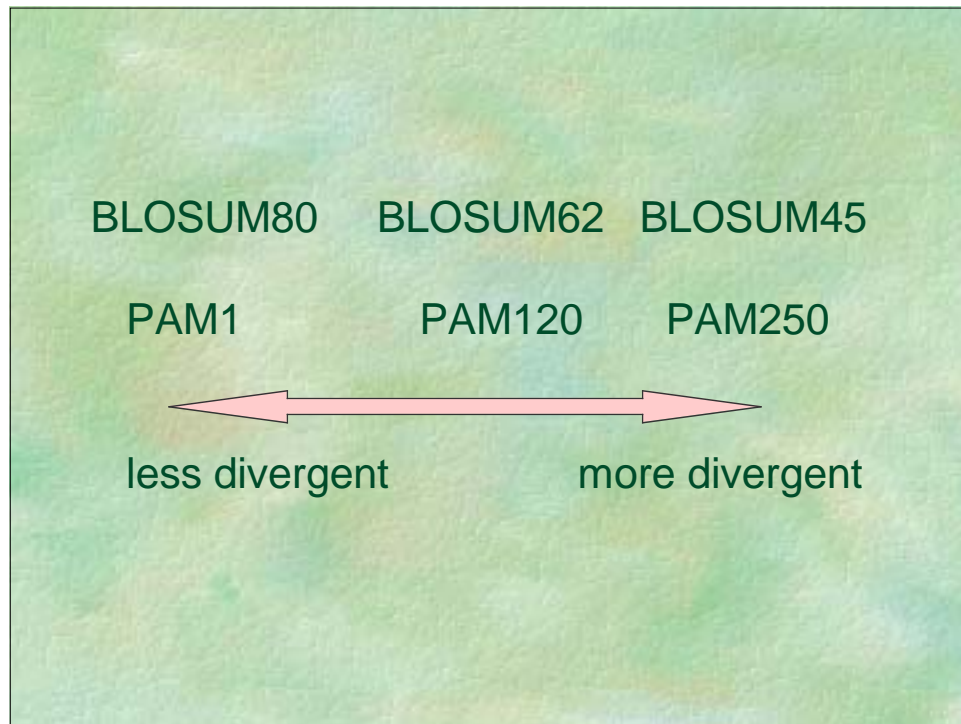
- ☛ Matrices are directly calculated, based on observed alignments
- ☛ Greater numbers are lesser distances
- ☛ Usually best for local similarity searches
- ☛ BLOSUM62= DEFAULT FOR BLAST.
If a distant relative, think about another matrix.

BLOSUM SCORING RULES

- ☛ Zero score means the frequencies of the pair in the database is that expected by chance
- ☛ A positive score means more frequent than chance
- ☛ Negative score means the pair is found less frequently than chance.

BLAST - Basic Local Alignment Sequence Tool

- ☛ Objective: find all local regions of similarity distinguishable from random
- ☛ Only local alignments permitted,
- ☛ Gaps permitted in version 2
- ☛ Statistically sound (Karlin and Altschul), but no guarantee of optimality



BLAST: Three Step Algorithm

- Compile a list of high scoring words of length w ($w=4$ for proteins, 12 for nucleic acids)
- Scan for word hits of score greater than threshold, T
- Extend word hit in both directions to find High Scoring Pairs with scores greater than S

Other BLAST Programs

- BLASTN: nucleic acid query to NA database
- BLASTP: Protein query to Protein database
- BLASTX: Translated nucleic acid query to Protein database
- TBLASTN: Protein query against (translated) nucleic acid database
- TBLASTX: Translated nucleic acid against translated nucleic acid database

OTHER BLAST VARIATIONS

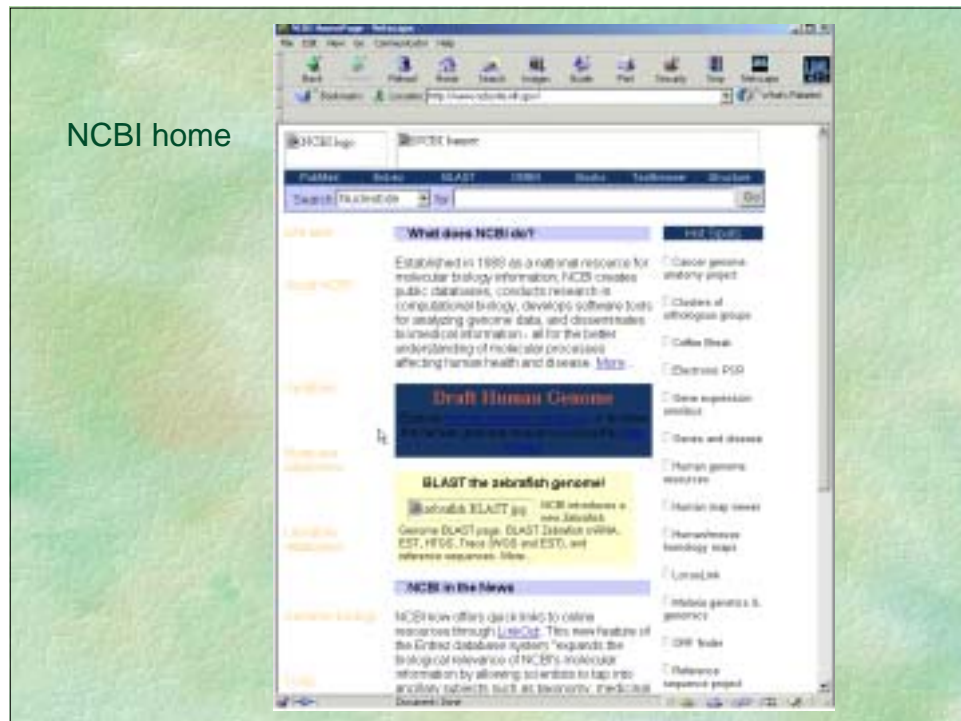
- Gapped BLAST (BLAST 2.0) -extend words from no-gap to gap, generate gapped alignments
- PSI-BLAST- Position Specific Iterated BLAST-use gapped BLAST, generate a Profile from multiple iterations used instead of the input and Distance Matrix

Limitations to BLAST

- ☛ Needs islands of strong homology
- ☛ Limits on the combination of scoring and penalty values
- ☛ The variants (blastx, tblastn, tblastx) use 6-frame translation-miss sequences with frameshifts)
- ☛ Finds and reports ONLY local alignments

A WALK THROUGH BLAST

NCBI home



NCBI *formatting BLAST*

Your request has been successfully submitted and put into the Blast Queue.

Query = (60 letters)

The request ID is

Format = **default**

The results are estimated to be ready in 30 minutes but may be done sooner.

Please press 'FORMAT!' when you wish to check your results. You may change the formatting options for your result via the form below and press 'FORMAT!' again. You may also request results of a different search by entering any other valid request ID to see other record jobs.

Format

Show ☒ Descriptions ☒ Links ☒ HITS ☐ Alignment ☐ HTML ☐ Cons

Number of Descriptions Alignment

Alignment size

Limit results by or select from (None)

Export table



BLAST RULES OF THUMB

- ☞ For short amino acid sequences (20-40), 50% identity happens by chance
- ☞ If A and B are homologous, and B and C are homologous, then A and C are, even if you can't see it.
- ☞ You can get similarity in the absence of homology for low complexity, transmembrane and coiled-coil regions. These have to be eliminated by you.

BLAST Significance

- ☛ If you change scoring systems, you can still compare search results if you normalize the score.

$S' = (\lambda S - \ln K) / \ln 2$. λ and K are associated with the scoring system.

S' , with a given E , is significant if it is greater than $\log N/E$, N the size of the search space.

FASTA: FAST Alignment

- ☛ <http://alpha10.bioch.virginia.edu/fasta/>

- ☛ <http://www2.ebi.ac.uk/fasta3>

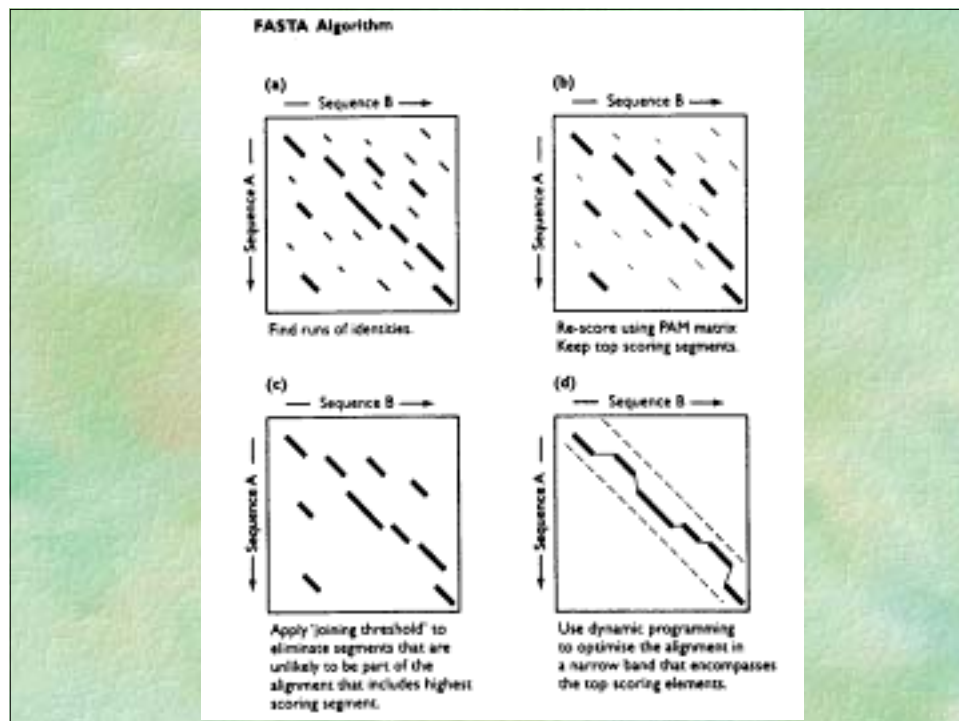
- ☛ <http://workbench.sdsc.edu>

- ☛ Rapid Global alignment

- ☛ Not a strong mathematical basis

FASTA: WHY USE IT?

☞ Allow alignments to shift frames



LALIGN

- Essentially a FASTA derivative for local alignments
- Compares two proteins to identify regions of similarity
- Will report several sequence alignments within a given sequence
- Works for internal repeats that are missed by FASTA because of gaps.

SITEs for LALIGN

- <http://fasta.bioch.virginia.edu/fasta/lalign.htm>
- <http://xylian.igh.cnrs.fr/bin/lalign-guess.cgi>
- <http://biowb.sdsc.edu> (registration necessary but painless)
- PALIGN
<http://fasta.bioch.virginia.edu/fasta/palign.htm>
(plots a graph of the areas of alignment)



ENTREZ: Linked Databases

<http://www.ncbi.nlm.nih.gov/Entrez/>

- ☞ Concept of Neighbor-usually BLAST relationship
- ☞ Precomputed=Fast
- ☞ Related sequence, structure neighbors, related articles

EST DATABASES:Quality issues

☞ SEQUENCE QUALITY

- calculated error less than 1% (Phred-20) is the rule
- frameshifts and stops common
- Rules are usually observed by exception
- There are lots of exceptions in the public data
- Many 3' UTRs

EST Databases: Quality #2

☞ CLONE QUALITY

- Over-representation
- Tissue specificity
- Developmental stage specificity
- Unprocessed mRNA clones
- Chimeras
- Contamination

EST Cluster Databases

- STACK-at SANBI <http://sanbi.ac.za>
- TIGR-animals, plants, other
<http://www.tigr.org/tdb/tgi.shtml>
- Unigene-NCBI
 - Human, mouse, rat, cow, zebrafish
 - mRNAs
 - predicted mRNAs



UNIGENE

☞ A LIST OF LISTS

- The cluster and known EST, mRNA pieces
- Additional annotation-gene name, etc.
- Distributed as a subset of dbest

NOT included in the BLAST searchable DB at NCBI

Caveats on Clusters

- ☞ Not stable
- ☞ Can go to complete cDNAs as available

LOCUSLINK

(<http://www.ncbi.nlm.nih.gov/LocusLink>)

- A useful, searchable compendium of loci across human, mouse, rat, Drosophila and zebrafish
- Linked for PubMed, OMIM, RefSeq, Homologene data, Unigene, and Variation Data

The screenshot displays the NCBI LocusLink web interface. The top navigation bar includes the NCBI logo and the LocusLink title. Below the navigation bar, there is a search bar and a list of genomic features. The main content area shows detailed information for the HLA-B gene, including its location on chromosome 6, its function as a class II histocompatibility antigen, and its association with various diseases. The interface is organized into several sections: Gene, Gene Structure, Gene Expression, Gene Variation, and Gene History. The Gene section provides a summary of the gene's structure and function. The Gene Structure section shows the gene's organization into exons and introns. The Gene Expression section displays the gene's expression profile across different tissues and conditions. The Gene Variation section lists the gene's associated polymorphisms. The Gene History section provides a timeline of the gene's discovery and characterization.

LocusLink provides links into:
 Pubmed,
 Omim,
 Refseq,
 HomolGene,
 Unigene,
 Variation
 data

A paper is currently in progress writing Haskok/Gene
conclusion and results

NCBI HomoloGene

Published | Draft | BLAST | CDS | Transcripts | Structure | Genome

Search: [id: 1000000000] [Go]

HOMOLOGENE ENTRY

Musculus HLA-D associated transcript 2 (Bat2)
 LocusLink | MGI | UniGene

POSSIBLE HOMOLOGOUS GENES

D. laurus ESTs, Highly similar to B55295 MHC class II histocompatibility antigen HLA-D associated protein 2 (refseq) - human (H30000)
 UniGene

H. sapiens HLA-D associated transcript 2 (BAT2)
 LocusLink | UniGene

R. norvegicus ESTs, Highly similar to Bat2, CNA segment, Chr 17, human D6S51E, RHEB1, CNA 311039605 gene (Mus musculus) (H. musculus)
 UniGene

CURATED ORTHOLOGS
 Published orthologs are reported in curated databases (RefSeq)

Ortholog	Homology	Ortholog
Musculus-Bat2	Human	H. sapiens-BAT2
	Mouse	
	Human	
	Mouse	
	Human	
Musculus-Bat2	Human	H. sapiens-BAT2

CALCULATED ORTHOLOGS
 Listed below are the nucleotide sequence comparisons used in determining homology. The % ID below includes hypotheses in the indicated alignments (RefSeq)

Organism-Gene	Sequence ID	% Sequence	Organism-Gene
Musculus-194_000027.00	100	100	R. norvegicus

Resources for Genomic Comparison

- GLASS-<http://plover.lcs.mit.edu>
- PipMaker: <http://bio.cse.psu.edu>
- Rosetta: [http:// plover.lcs.mit.edu\(genes\)](http://plover.lcs.mit.edu(genes))
- SGP: <http://soft.ice.mpg.de/sgp-1>
- VISTA: <http://www-gsd.lbl.gov/VISTA>
- WABA:
<http://www.cse.ucsc.edu/~kent/xenoAli/index.html>

EFFICIENT SEARCHING

☞ Use Wild Cards: #,\$,?,*

☞ Use Boolean Operators

- Not
- And
- Or
- Nor

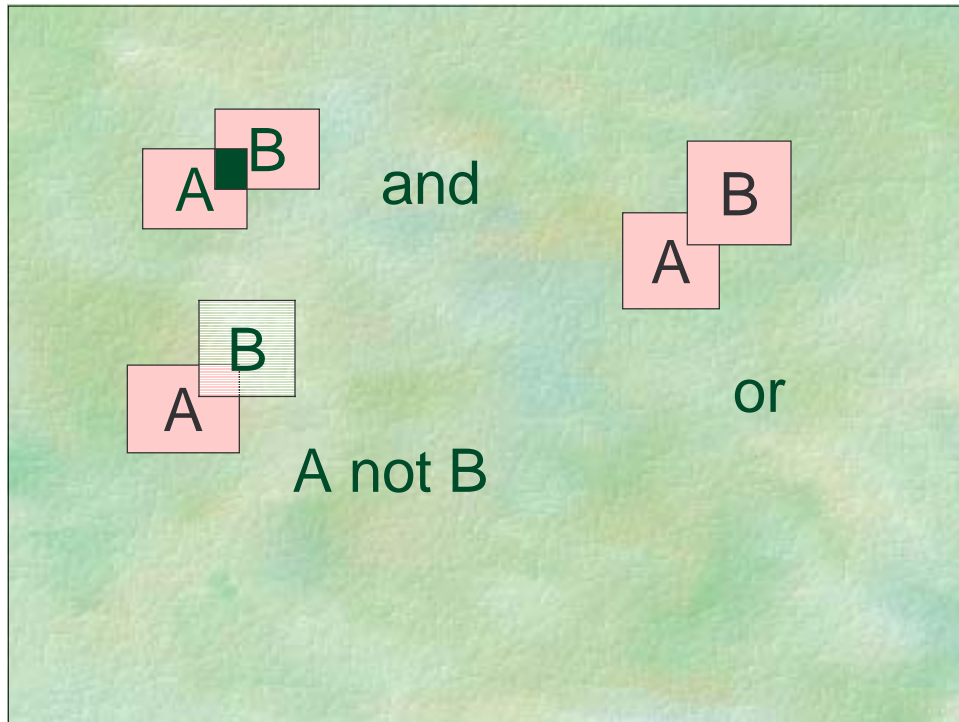
Boolean Operators

☞ *AND* A and B BOTH

☞ *OR* A or B EITHER

☞ *NOT* B not A Have B, do not have A

☞ *NOR* A nor B A but not B OR B but
not A



WILD CARDS

- ☞ Match one character-NCBI uses #
- ☞ Match zero or one character NCBI uses \$, others ?
- ☞ Match zero or more characters-usually *

RULES OF THUMB

- ☞ Use an up-to-date database; repeat often
- ☞ Choose a fast algorithm
- ☞ Use the most recent version
- ☞ Work at the protein level--for a small amount of evolutionary change, DNA sequence contains less information about homology
- ☞ Respect your own *intuition*

Other Resources

- ☞ NCBI Education Page
<http://www.ncbi.nlm.nih.gov/Education/index.html>
- ☞ BCM Gene Finder
http://searchlauncher.bcm.tmc.edu/docs/sl_links.html
- ☞ EBI-SwissProt, TrEMBL, PIR, SRS, Tools
<http://www.ebi.ac.uk>
- ☞ ExPASy-SwissProt, TrEMBL
<http://www.expasy.ch/>
- ☞ DISC-DNA Information and Stock Center
<http://www.dna.affrc.go.jp>

MEDICAL SUBJECT HEADINGS

- ☛ CONTROLLED Vocabulary
- ☛ Indexing of articles, books, etc.
- ☛ Current version has over 300,000 terms
- ☛ Can download list and make your own assortment

MeSH Advantages

- ☛ Assigned to the the entire document, not just title and abstract
 - ☛ Major topic (*)
 - ☛ Subheadings if available
 - ☛ MeSH topics are exploded to include all the terms included in the meaning.
- Try it; you may like it.

